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THE HISTOLOGY OF THE LIGHT ORGANS OF PHOTINUS MARGINELLUS.¹

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ORGANIC light has always been a subject of interest, both to the scientist and to the casual observer of nature, and no other photogenic organisms are of such wide range and easy access as the fireflies. In some of its phases the light of the firefly comes within the scope of the physicist and chemist. To the former belongs the consideration of its nature, of its spectrum and other physical properties ; to the latter come the problems of chemical analysis concerned with the phenomenon, but to the student of entomology remains the study of the delicate living structure in which this wonderful process of photogeny takes place.

In spite of the abundance of these insects, little work has been done on the structure of the light-organs in our American fireflies. During the past twenty years some careful investigations have been made upon European and Cuban forms, but there is practically no literature upon those of our own country. The purpose of my study has been to learn something of the histology of the light-organs of *Photinus marginellus*, the most common firefly about Ithaca during June and July. This work was begun at the suggestion of Dr. Wm. H. Seaman of Washington, D. C., whose advice during its progress has been most helpful. I am also indebted to Professor Comstock and to the members of his staff at Cornell University for their kindly supervision of my work.

The material from which this work was done was collected near Cornell University during the summers of 1901 and 1902. The insects begin to appear by the middle of June, but are not abundant until July. The height of the flying season is during the first part of July. The large majority of the insects

¹ Contribution from the Entomological Laboratory of Cornell University.

caught were males. A number of females were found, but never on the wing. They were always either in the grass, or on some low plant where they could easily have climbed.

Three methods of fixation were tried the first summer. For two of these the insects were killed by dropping into hot water. The caudal part of the abdomen was then clipped off and put into the fixing fluid without removing any part of the chitin. Half of this material was fixed for twenty-four hours in Flemming's solution (strong). This proved almost worthless, with the exception of a single slide. The other half of this material was fixed in Gilson's mercurio-nitric solution for seven hours. This gave very good preparations of the general features of both layers of the light-organs.

The third lot of material was killed and fixed in hot 70% alcohol. This was satisfactory for the gross structure of the organs, though not good for the finer details.

The second summer the killing of the insects in hot water was abandoned. The cadual portion of the abdomen was clipped off, the tip cut away, and the dorsal wall removed to insure better penetration of the fixing fluid. The living tissue was then put directly into the fixer. Flemming's fluid, after this treatment, gave much better results than before. Hermann's fluid gave a fairly good fixation. The cells of the cylinders are definitely outlined in these preparations, but the tissues in general are opaque.

By far the best results for the tracheal structures were obtained by the use of osmic acid. The strength of the acid was varied from .1% to 1%, and the time of fixation, from two to thirty hours. The best preparations were from material left for thirty hours in 1% osmic acid.

Material fixed in Flemming's or Hermann's fluid, or in osmic acid, was washed for twenty-four hours in running water. That fixed in Gilson's fluid was put directly into 70% alcohol. All the material was dehydrated by carrying it through the grades of alcohol, from 70% to absolute. Cedar oil was used for clearing. These sections were all cut in paraffin.

The greater part of the sections were cut 10 μ in thickness, but a few thinner sections, 3 μ and 5 μ , were made for the determination of some finer structural points.

The material was all stained after the sections were cut. The Gilson and alcoholic sections were stained for about two minutes in Gage's chloral hematoxylin, and for a half minute in eosin. This gave satisfactory general results. A double stain with alum carmine and picric acid did not prove a good stain after Gilson's fluid. Following fixation with osmic acid, Hermann's or Flemming's fluids, safranin proved most satisfactory. The sections were stained for twenty-four hours in a mixture of equal parts of saturated aqueous and alcoholic solutions of safranin, then differentiated in absolute or even slightly acid alcohol. Iron hematoxylin is also a good stain for osmic acid material.

Carbol-xylene was used as a clearer, and the sections were mounted in Canada balsam.

Teazed preparations were made by dissecting out the light-organs entire, placing them for from fifteen to thirty minutes in .5% osmic acid, then for a few minutes in weak caustic potash, and teasing in normal salt solution.

Experiments with methylene blue injection were not successful. Only one insect showed any coloration of the central nervous system, and in that the finer nerves could not be traced. It is difficult to get a good injection with such small insects. However, my attempts in this line were begun near the close of the collecting season, and with further experimenting it is possible the results might have been better. The most successful specimen was killed an hour and a half after injection.

Since the old idea that the firefly's light was dependent upon the presence of phosphorus or some similar substance has been abandoned, other theories have been advanced in attempt to explain the phenomenon. The view generally accepted is that the light results from the oxidation of a substance produced by the metabolism of the light-organ cells. The nature of this substance has not been determined, but that its photogenic property is independent of the life of the cell is proved by the fact that when the organs are dried and reduced to a powder the light reappears under the influence of air and moisture. When the fresh photogenic tissue is crushed, the light increases in brilliancy, and it is some time before it wholly disappears. Radziszewski ('80) through his study of the artificial luminosity

of lophin, discovered a series of carbon compounds similar to those found in living organisms and capable of becoming luminous under conditions compatible with life. The conditions necessary for this production of light he found to be the presence of oxygen, an alkaline reaction and slow chemical action. Watasé ('96) states that in the firefly the phenomenon is due to the oxidation, in alkaline media, of a granular substance secreted by the cells of the photogenic tissue. He offers no further suggestion as to the character of the substance than that it is "a secretion of fatty nature." He gives as proof of the oxidation theory the fact that when the photogenic material is crushed on a slide and lowered into a jar of carbon dioxide the light disappears instantly, but reappears when the slide is placed in a jar of oxygen, or simply in the air. This may be repeated several times with the same material. Watasé recognizes the necessity of moisture as well as of oxygen in the process of photogeny.

Dubois stands almost alone in opposing the theory of oxidation. As a result of experiments with ozone, nascent oxygen and oxygenated water he states ('95), that the action of energetic oxidizing reagents at once and finally extinguishes the light, without first causing any increase in brilliancy. However, the absence of oxygen seems to destroy the light, as it is suspended when the light-organs are placed in a vacuum. From his earlier work he concluded that the light was the result of a process of crystallization. His later work ('98^a) has led him to abandon this theory. He still rejects the oxidation hypothesis as crude and unscientific, and offers in its place one of a reaction between two substances to which he has given the names luciferase and luciferine. The accessibility of the material led him to use *Pholas dactylus*, a marine mollusk, as the basis of his study. The inner wall of the siphon of a large *Pholas* was scraped with a knife and the resulting pulp crushed with sand and 95% alcohol. After twelve hours it was filtered and a liquid obtained which was not luminous, even after vigorous agitation with air. The alcohol was drained off from the residue and chloroform added. After some hours a second non-luminous liquid was filtered off. A mixture of one part of the first liquid with three parts of the second gave a beautiful

luminescence at ordinary temperature. By adding to liquid No. 2 five or six times its volume of 95% alcohol, or by boiling it, a white floccose precipitate was formed and the mixture of the remaining liquid with liquid No. 1 no longer produced light. He therefore considers the white precipitate as constituting one of the two photogenic substances, the luciferase. Luciferine was obtained in an impure state by evaporation of alcoholic liquid No. 1. Another experiment was tried with the luminous mucous secreted by *Pholas*. Two portions of the mucous mixed with water were taken, and one extinguished by agitation, the other by bringing to the boiling point. The mixture of the resulting non-luminous liquids was photogenic. A similar result was obtained with the prothoracic organs of *Pyrophorus noctilucus*. One was extinguished by crushing, the other by dropping it into boiling water. When the latter was crushed and mixed with the former, the light reappeared.

Dubois therefore states that he has established experimentally that the light of living organisms is produced, in the presence of water and oxygen, by the reaction between luciferase, an instable proteid substance possessing in large measure the general properties of an enzyme, and luciferine, a chemical substance. While Dubois confidently asserts that biological light is not a result of oxidation, his experiments would not seem to prove this conclusively. He himself admits the necessity of oxygen, and even if the process is not one of simple, complete oxidation, it would yet seem probable that oxidation is the essential factor in photogeny.

The photogenic tissue of *Photinus* responds definitely to the action of oxygen. A series of experiments shows uniformly a decided increase in the brilliancy of the light when the tissue is placed in a jar of oxygen. Tissue in which the light has been wholly extinguished by the action of carbon dioxide becomes instantly photogenic when placed in oxygen. Until there has been more extensive experimental study of a large number of organisms, generalizations on the subject of organic light are unsafe.

The fact that Dubois's work was done upon a marine organism is significant. If, as one must assume, the photogenic function

has arisen independently in different organisms, it would not seem strange that the light of such widely separated forms as a marine mollusk and a terrestrial insect, though in both cases a process of oxidation, might be produced in a different manner.

The physical properties of the firefly's light have been studied by Dubois, Langley and Very, Young, and Watasé, with essentially the same results. The spectrum given by the light of the *Lampyridæ* is perfectly continuous, without any trace of lines, either bright or dark. It lies within that portion of the spectrum which most powerfully affects the organs of vision, though having small thermal or actinic effect. Dubois has demonstrated by photography the presence of some actinic rays in the light of *Pyrophorus*. A single insect was used, and five minutes was required for printing from a plate which would have taken only a fraction of a second with sunlight. Dubois attributed the presence of actinic rays to a fluorescent substance which he found in the blood.

Most careful and elaborate experiments have failed to show more than an infinitesimal amount of heat connected with the light. One authority even goes so far as to say that not more than one-thousandth of the energy expended in the flash of the firefly is converted into heat waves. When one considers that in our ordinary oil or gas lamps more than ninety-nine per cent of the energy is lost as regards illumination, and that even in the arc light only about ten per cent. of the waves are visible, the interest which this "cheapest form of light" arouses from the economic point of view is very apparent. It is also an alluring problem to the student of physics to determine by what process the medium wave lengths are produced independent of the longer and shorter waves. If this "secret process" could be wrested from nature, its economic value would prove almost inestimable.

While the phenomena of biological light early attracted the attention of observers of nature, as Aristotle, Democritus and the naturalist Pliny, it is only within the last century that any serious study has been given to the organs which produce it. The discovery of their cellular nature may be credited to Peters. In 1841 he refuted the theory of Carrara ('36) that the light was dependent upon an air-sac extending from the mouth to the light-

organs and acting as a bellows, and stated that the photogenic tissue was made up of little spheres, regularly arranged and penetrated by the tracheæ. Leydig and Kölliker in 1857 definitely recognized the cellular structure of the light-organs. Their work has been followed by that of several other European investigators. During the past twenty years Wielowiejski and Emery have made important histological researches upon species of *Lampyrus* and *Luciola*.

All recent workers agree in stating that the ventral light-plates of the male lampyrids are composed of two more or less clearly defined layers; the dorsal, chalky, opaque layer, and the ventral, or truly photogenic layer. The former is composed of fairly regular, polygonal cells, filled with a great quantity of crystals of urate salts. The ventral layer is composed of two distinct elements; the tracheal structures and intermediate areas of parenchyma. The parenchyma cells contain fine granules of non-urate composition. The main tracheæ of the photogenic segments send vertical branches down through the light-organs. Aside from their profuse branching they show no unusual features until they reach the ventral layer. The tracheal structures within the ventral layer differ in different forms, and the two species upon which Wielowiejski and Emery based the bulk of their work, *Lampyrus splendidula* and *Luciola italica* respectively, show a considerable difference in this respect. In *Luciola* each vertical tracheal axis is surrounded by a cylindrical mass of semi-transparent tissue, within which it branches in an arborescent manner. The method of branching in *Lampyrus* is fasciculate, rather than aborescent, and the tracheæ are much less regular in their distribution. Max Schultze, in 1864, found in osmic acid preparations from the light-organs of *Lampyrus splendidula* certain blackened bodies at the periphery of the cylinder. These he found to be penetrated by the finer tracheæ. Failing to find further continuations of the tracheæ beyond these bodies, he called them the "tracheal end cells." Wielowiejski ('82), in his study of the same species, found that instead of having their ultimate endings within the so-called "end cells," the tracheæ branch, sending out fine "tracheal capillaries" which extend beyond the cylinder and in most cases anastomose with those of

adjoining cylinders. Emery, from his work on *Luciola*, confirms the views of Wielowiejski in all points except as regards the anastomosing of the capillaries. He found them always ending free, never uniting with those from the same or another cylinder.

In this work the nomenclature previously used has been retained except in a few cases where a change seemed especially advisable. The more familiar terms phosphorescent and luminous, with their nouns, are abandoned and photogenic and photogeny substituted. Phosphorescent is objectionable as it suggests that the light is due to the presence of phosphorus. Photogenic — light-generating — gives a more definite idea of the actual phenomenon than luminescent. The name "end cells" was used by Max Schultze because he believed the tracheæ had their ultimate endings within these cells. Now that it is proved that the tracheæ do not so end, but merely pass over into the tracheolar network, the name "transition cells," as used by Holmgren ('96), is far preferable. The term tracheoles, which is used elsewhere in insect histology to designate fine tracheal branches not possessing spiral thickening, is preferable to "tracheal capillaries."

The light-organs of the male of *Photinus marginellus* are in the form of two plates lying above the ventral body wall of the

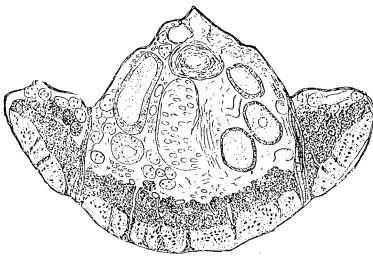


FIG. 1.—Transverse section through entire abdomen. $\times 20$.

fifth and sixth abdominal segments. (Figs. 1 and 2.) The underlying cuticle is transparent, allowing free emission of the light. The plates lie just beneath the central nervous system and directly upon the very thin hypodermis. They are yellowish white in color. In the female of the

same species there is only a single, somewhat spherical organ in the centre of the fifth segment. In this work all descriptions of structure refer to the light-organs of the adult male. The main tracheæ of the photogenic segments send branches ventrad through the light-organs. Thus the dorsal surface in fresh

material is shown to be pentetrated by numerous tracheæ. The vertical or oblique tracheæ continue to branch profusely in an arborescent manner. This repeated branching is characteristic of the tracheæ of the photogenic tissues. The tracheal epithelium is composed of thin, flattened cells, with large flattened nuclei. Prominent hair-like projections of the intima are abundant in the lumen of the large tubes. These internal chitinous hairs have been noted in *Lampyris* by Gerstæcker, and in *Luciola* by Emery. The light-organs are innervated by nerves from the last two abdominal ganglia. These ganglia are both situated in the fifth segment, over the more cephalic light-plate. I failed to trace more than these primary nerve branches, as my attempts at methylene blue injection were unsuccessful.

Each light-plate is composed of two distinct layers, in this

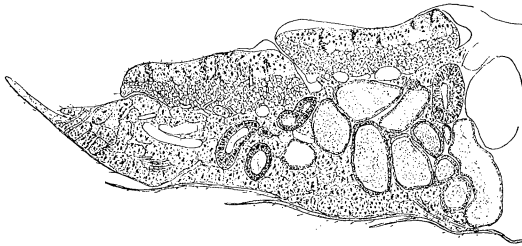


FIG. 2.—Longitudinal section through abdomen from fifth segment to caudal end. $\times 20$.

respect agreeing in structure with the European species which have been studied. In none of my preparations have I been able to detect any trace of a membrane, either surrounding the light-organs or separating the two layers. Wielowiejski ('82) states that in *Lampyris splendidula* each light-plate is surrounded by a delicate film of connective tissue, in which small rounded nuclei may be faintly seen in well stained material. He gives no figures of this, however. Emery ('84) says there is no indication of a membrane in *Luciola*. The two layers cannot be distinguished in fresh material, but a difference in the two surfaces of the light-organ is apparent. The dorsal surface is a bright chalky white, while the ventral surface appears yellowish and luminous. Examined with a low power of the micro-

scope the dorsal surface is seen to be divided into polygonal areas.

The appearance of the cells of the dorsal layer varies much with the treatment of the tissue. Material fixed in alcohol and brought in contact with water for but a short time in staining with hematoxylin shows the cells filled with a dense content of coarse granules. With reflected light these granules still show their characteristic chalky whiteness, while with transmitted light they are brown. Granules identical in appearance are also found in the fat cells of the same region of the body. Material fixed in any fluid requiring subsequent washing in water shows

a considerable decrease in the granules of the dorsal layer. This verifies the statement of Wielowiejski that these granules are insoluble in alcohol, but soluble in water. Kölliker, in 1857, proved them to be crystals of urate salts, and his results have been accepted by Wielowiejski and Emery. When the crystals have been dissolved out the form of the cells is easily determined.

They are polygonal, fairly regular in outline and similar

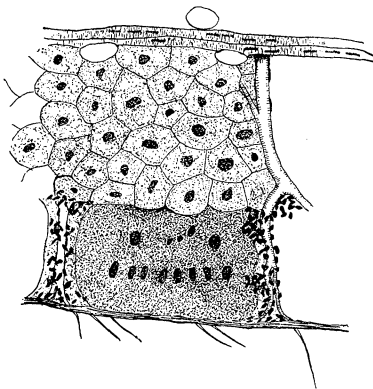


FIG. 3.—General features of both layers of light organ. Gilson's fluid, hematoxylin and eosin. $\times 75$.

in size. They average about $28\ \mu$ by $25\ \mu$. Those upon the upper surface are somewhat more spherical than those beneath. Large nuclei are always present, but the cytoplasm seems to have been almost entirely replaced by the granular secretion. (Fig. 3.)

The dorsal layer not only forms a plate resting upon the ventral layer, but it projects beyond the latter and extends along its caudal surface to the body wall (Fig. 2). There are two groups of muscle fibres in each light plate, extending from the dorsal to the ventral body wall in the lateral portions of the plate. These muscles are surrounded by a layer of cells distinctly separated from the cells of the ventral layer and contin-

uous with those of the dorsal layer. In material in which the dark granules of the dorsal layer cells have not been dissolved out, they are found equally in the cells surrounding these groups of muscle. (Fig. 1.)

The ventral layer is composed of two distinct elements, the so-called parenchyma cells, and the cylinders. The cells of the parenchyma differ from those of the dorsal layer in being very irregular in shape and size. Occasional cells extend from the dorsal surface of the layer to the body wall.

In some cases the depth is several times the thickness, while some cells are almost spherical. In some places the ventral layer is found to be several cells deep. (Fig. 4.) The size of the cells varies considerably. The granular secretion in these cells is much finer than that in the dorsal layer. Max Schultze has stated that these granules are of non-urate composition. He examined the granules of both layers with polarized light, finding that those from the dorsal layer were bi-refractive, but that those from the ventral layer were not. Those of the dorsal layer having been proved by Kölliker to be of urate composition, he concluded the granules of the ventral layer were non-urate. Wielowiejski verified the results of these experiments, but did not agree with Max Schultze's conclusions. He states that these experiments merely prove the granules of the ventral layer to be in general amorphous. He thought, however, that the difference in composition could be readily demonstrated by reagents. He found the granules of the dorsal layer to be soluble in water but insoluble in alcohol, while the opposite was true of those of the ventral layer. Emery says that the granules of the ventral layer are not crystalline, and that they disappear altogether in balsam preparations. The cells of the ventral layer appear to have more or less of granular content in all of my preparations, including those from material fixed in alcohol. It is only in osmic acid material that cells are found comparatively free from such secretion. As all my sections are mounted in balsam, this medium would not seem



FIG. 4.—Cells of parenchyma. .3 % osmic acid. $\times 250$.

to have any decided effect. This question, however, can be satisfactorily settled only by the study of fresh tissue.

Between the areas of parenchyma are sharply defined, more or less cylindrical masses of tissue surrounding the vertical tracheal stems and their branches. It is about these cylinders and their tracheæ that the greatest interest is centered. The cylinders are from $23\ \mu$ to $68\ \mu$ apart, and average about $30\ \mu$ in diameter. Their appearance differs greatly with the fixation. In material fixed in alcohol or Gilson's fluid, and stained with hematoxylin and eosin they appear as areas less granular, and consequently less deeply stained, than the intervening parenchyma. They contain a large number of small nuclei, especially abundant near the tracheæ. After fixation in Hermann's fluid and staining with safranin the cell outlines appear very distinct. (Figs. 10 and 11.) Preparations fixed in Flemming's solution and stained with safranin also show cellular structure, though not so definitely as the preceding. Less indication of the structure of the cylinders is shown in the osmic acid material.

If fresh material, placed for fifteen minutes in .5 % osmic acid and then treated for a few minutes with weak caustic potash, is viewed from the ventral surface, the cylinders appear as very distinct rings. In all preparations, both temporary and permanent, the boundaries of the cylinders are sharply defined.

Within each cylinder is a main tracheal stem which gives rise to numerous branches in the characteristic aborescent manner. There is no change in the structure of the tracheæ until near the periphery of the cylinder, where each fine tracheal twig breaks up into tracheoles. The number of tracheoles arising from one tracheal twig seems to vary somewhat. Ordinarily there appear to be only two, but three or four are not uncommon. Emery gives the number of tracheoles in *Luciola* as being uniformly two. In *Lampyrus*, Wielowiejski found the number variable, as many as six sometimes occurring.

The tracheoles are fine tracheal branches and are characterized by having no spiral thickening of the intima. Their chitinous structure is plainly shown by the fact that they persist in material treated for some time with caustic potash.

Max Schultze, Targioni-Tozzetti ('70) and Emery were all of

the opinion that the tracheoles do not contain air, but a colorless fluid. Wielowiejski also found them filled with a fluid, but recognizing the extreme improbability of such condition existing in life, he looked for some explanation of it. In dried, air-filled material mounted in weak glycerine the tracheoles as well as the larger tracheal tubes were filled with air. After about five minutes the silvery, glistening lines of air became broken up,

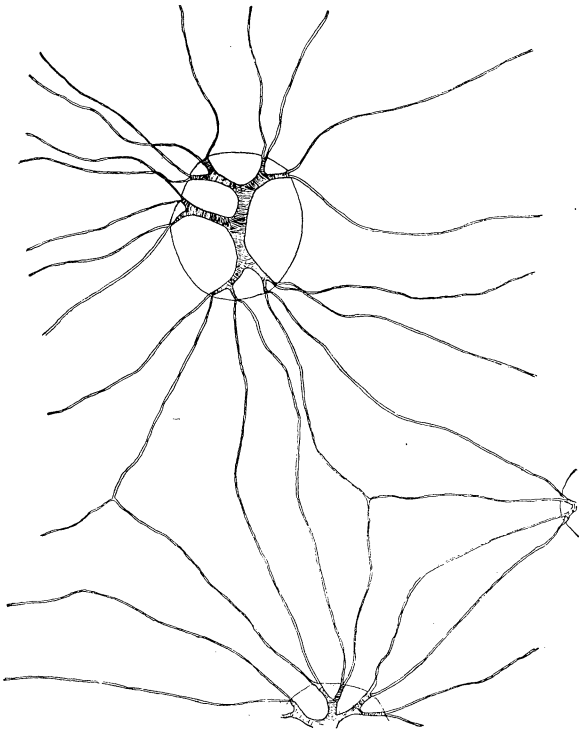


FIG. 5.—Teased preparation, showing anastomosing of tracheoles.

and gradually, from the tracheoles in, the tracheæ became filled with a fluid. This would seem to prove, what one must believe *a priori*, that the entire tracheal system is filled with air. My observations agree with Wielowiejski's, for although in my preparations the tracheoles were always already filled with liquid, the penetration of the liquid into the larger branches was unmistakably from the tracheoles in. It may be noted in this con-

nection that in the tracheoles of developing wings, structures entirely similar to those of the light-organs, the presence of air may be readily seen.

In sections parallel to the axis of the cylinder the tracheoles are generally cut, so that their entire length cannot be followed. The fact that they appear to end free cannot, therefore, be taken as any proof. In rare cases they are seen to anastomose with those of adjacent cylinders, and in sections transverse to the cylinders, beautiful demonstrations of anastomosing may be seen. If a light-organ from a freshly killed insect is placed for a half hour in .5 osmic acid, then for a few minutes in caustic potash, and then placed under the microscope, ventral side up, the tracheoles can be easily seen. The cylinders stand out as definite circular or oval rings, and from the inclosed tracheæ radiate the tracheoles. As the distribution of the cylinders is fairly regular, the network of tracheoles has a notably uniform pattern. Tracheoles from three cylinders unite at a point about equidistant from their respective cylinders. (Fig. 5.) In preparations from material fixed in Flemming's, or Gilson's fluid, the same tracheolar network may be clearly seen. (Fig. 6.)

When the ventral surface of a fresh light-organ is studied under the microscope in a dark room the light is found univer-

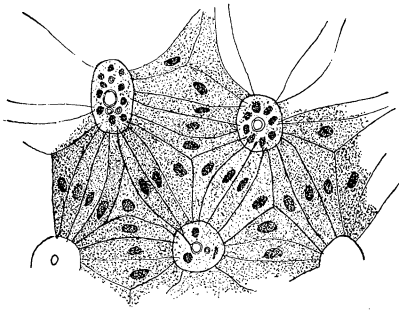


FIG. 6.—Anastomosing of tracheoles. Fleming's fluid, safranin. $\times 150$.

sally distributed throughout the parenchymatous area. The cylinders stand out as non-photogenic spots on the background of light. This shows that the photogeny occurs in that portion of the tissue where the tracheolar network is found, and where there is consequently the most abundant supply of oxygen.

Wielowiejski found anastomosing of the tracheoles generally true in *Lampyrus*, although he admits of some exceptions to the rule. Wistinghausen and Holmgren found anastomosing of the tracheoles in the silk-glands of the caterpillar. Emery states

that in *Luciola* the tracheoles in all cases end free. This is shown in his figures 4 and 7, Plate XIX.

In *Lampyrus* Wielowiejski figures the tracheoles as winding irregularly and twisted and looped about each other. In *Photinus* they are generally almost straight. They may be slightly wound about each other, but for the most part they pass directly from one cylinder to another.

Both Wielowiejski and Emery agree in considering that the tracheoles pass between the cells of the parenchyma, although positive proof is difficult. In no case has any portion of a tracheole been found within the parenchyma cells, although they have been seen closely applied to the exterior of the cells. In surface sections of *Photinus* the areas between the tracheoles would appear to correspond to cells, each possessing a nucleus. It thus seems altogether probable that in their course outside the cylinders the tracheoles are intercellular.

In 1864 Max Schultze studied the light organs of *Lampyrus splendidula*, using osmic acid as a fixer. He found the finer tracheal branches losing their spiral thickening and passing into star-shaped, finely granular bodies which he believed to be true cells possessing distinct nuclei and cell membranes. He failed to find any continuation of the tracheæ beyond these stellate cells, and so assumed that these cells enclosed the ultimate endings of the tracheæ. The name "tracheal end-cells," as given by Max Schultze has since been generally used, even by those who knew it to be a misnomer.

In his studies of the same species, Wielowiejski found the tracheæ passing into the stellate "end-cells" of Max Schultze, but instead of ending there, branching to form tracheoles which penetrate the inter-cylindrical parenchyma. He believed these "end-cells" to be true cells, much flattened and similar in form to the endothelial cells of vertebrates. They extend about the bases of the tracheoles in a web-like manner and are more or less stellate in shape. These cells show a characteristic reaction with osmic acid, causing a precipitate to be formed, especially about the point of origin of the tracheoles. The tracheal twig appears constricted at its apex, and is intensely blackened by the osmic acid. The effect of the acid varies, in some cases the entire end cell being uniformly blackened.

In the female of *Lampyrus noctiluca* and the female and larva of *Lampyrus splendidula* Wielowiejski found a somewhat different condition than in the adult male of *L. splendidula*. The tracheoles arising from the ends of the finer tracheæ are generally only two in number. They may also occur along the course of the smaller tracheæ, instead of only at the ends, and they may even arise from some of the larger tracheal branches. It is obvious that under such conditions "end-cells" like those figured for *L. splendidula* would not be present. He states, however, that there is a membrane spread out between the tracheoles, although it fails to give the characteristic "end-cell" reduction with osmic acid. From the larva of *L. noctiluca* he figures one of the larger tracheæ with its branches, with a strongly developed, nucleated membrane surrounding it much as a cylinder surrounds the tracheal axis in the imago of Photinus. The epithelium of the large primary trunks of the tracheæ in the larva shows the power of precipitating osmic acid.

Wielowiejski also studied tracheal endings in other parts of the adult *L. splendidula*. In the fat body and reproductive organs he found "end-cells" in abundance, similar to the typical ones of the light-organs in their reaction with osmic acid, but differing considerably in shape and in the number of tracheoles contained. In all these instances Wielowiejski interprets the "end-cells" as being a special development of the epithelium of the trachea. In his figures the tracheæ, before entering the photogenic tissue, show well developed epithelium, this layer being sometimes almost as thick as the diameter of the tube.

Wielowiejski neither figures nor describes cylinders in the light-organs of Lampyrus. After his study of *Luciola italica* ('86) he states that he found no such regular arrangement of the tracheæ here as occurs in the two species of Lampyrus formerly studied. He did not consider the mass of the cylinders in *Luciola* as homologous with the "end-cells" of Lampyrus.

In *Luciola italica* Emery found the ventral layer composed of cylinders and intermediate areas of parenchyma much as has been already described for Photinus. In osmic acid preparations he saw, just within the periphery of the cylinder, small, irregular, three-cornered masses, in which the distal ends of the tracheæ

and bases of the tracheoles appeared to be imbedded. When a tracheal axis was isolated these small bodies looked like "grapes on a stem," while from each "grape" two tracheoles proceeded. As these browned bodies were found only in the osmic acid preparations he believed them to be artifacts, and not the "tracheal end-cells" of Max Schultze. He concluded that the clear cell elements of the cylinder are the real "end-cells." Within these the tracheæ undergo their final division, each giving rise to two tracheoles. In *Luciola* only that part of the cell which is in direct contact with the bases of the tracheoles is blackened by osmic acid.

Emery agrees with Wielowiejski in considering that the "end-cells" are formed from the tracheal epithelium.

Two of the latest investigators of tracheal endings, Wistinghausen and Holmgren, have both worked on the silk-glands of lepidopterous larvæ. Both found the finer tracheæ passing over into what they term the "tracheal capillary end-network," a network formed by the anastomosing of the tracheoles and their branches. They agree in stating that the epithelium of the tracheoles is extended in a web-like manner to form the "end-cells." Holmgren discards the term "end-cells," substituting for it the more correct name of "transition cells," as these structures form the transition between the tracheal tubes proper and the tracheolar net-work.

In the light-organs of *Photinus*, fixed for thirty hours in .1 % osmic acid and stained with safranin, the transition cells may be seen most plainly. They show with varying

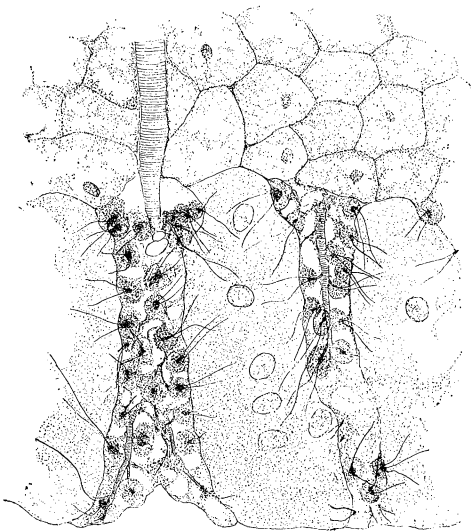


FIG. 7.—Transition cells in typical osmic acid preparation. $\times 250$.

clearness in all the osmic acid material, and in one insect fixed in Flemming's fluid. In the typical osmic acid preparation they appear as irregularly spherical bodies, blackened throughout but most intensely so at the point of origin of the tracheoles. They show no appearance of nuclei, but as the nuclei of the adjacent cells show only faintly with this treatment, this is not significant.

The transition cells of *Photinus* as shown in osmic acid preparations are more similar to the blackened, grape-like bodies described by Emery, than to the stellate, endothelioid cells figured by Wielowiejski. They occur at the apices of the finer

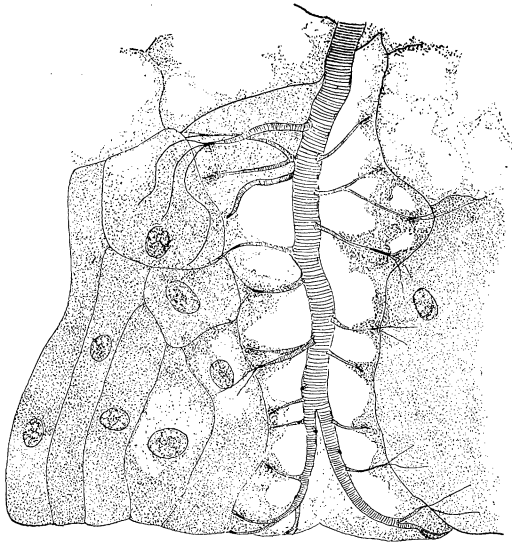


FIG. 8.—Transition cells where effect of osmic acid has been less pronounced. $\times 300$.

tracheal twigs, and near the periphery of the cylinder. The space between them and the tracheal axis, and the spaces between the transition cells themselves appear clear. The edges of the spheroid masses are generally irregular, and their whole appearance suggests an artificial condition. (Fig. 7.) In sections where the effect of the osmic acid has not been as extensive, the same blackening at the points of tracheolar origin may be seen, but instead of finding spherical bodies surrounding these points of furcation, the granular mass of the cylinder appears in different condition. It extends along the periphery of the

cylinder, and follows the course of each tracheal branch, in some cases almost to the main stem, so that instead of a structure resembling a cluster of grapes, one finds along the wall of the cylinder a series of fan-shaped masses, one for each tracheal twig, their apices toward the axis of the cylinder. (Fig. 8.) As there is great irregularity in the form of these dark bodies within the cylinder, and also in the shape of the intervening clear spaces, it would seem that Emery is correct in considering them an artifact.

Definite cellular structure can be seen only in the material fixed in Hermann's and Flemming's fluids. In these preparations, as in those from osmic acid, the tissue seems to be shrunken and distorted. To a large extent the nuclei appear to have been separated from the cytoplasm, and to lie in the spaces left by the shrinkage of the cells. The cells show a tendency to shrink away from each other, and away from the main axis of the trachea, thus becoming smaller, denser bodies surrounding the distal ends of the tracheal branches, and in contact with the periphery of the cylinder. (Figs. 9 & 10.)

In material fixed in alcohol or Gilson's fluid there is no appearance of cells within the cylinder, although an abundance of small nuclei may be clearly seen.

Emery suggests that the cylinder, in *Luciola*, may be a syntetium, but in both longitudinal and transverse sections of the cylinders in material of *Photinus* fixed in Hermann's fluid, the cells are clearly demonstrated.

The "end-cells" of *Lampyris* and the cylinders of *Luciola* are stated to be a special development of the tracheal epithelium. This is not true of the cylinders in *Photinus*, as the epithelium can be definitely seen, not only in the tra-



FIG. 9.—Section parallel to axis of cylinder. Herman's fluid. $\times 235$.

cheal axis, but even in the small branches. (Fig. 11.) The epithelium of the tracheæ of the photogenic tissue is altogether different from that figured by Wielowiejski for *Lampyris*. Instead

of being thick, it is very thin, with large flattened nuclei. In both longitudinal and transverse sections, where a nuclear stain has been used, the epithelium may be readily traced within the cylinders, even to the finer branches. It is exactly similar in appearance to that in the dorsal layer, and is wholly distinct from the cellular elements of the cylinder. Small flat nuclei are seen closely applied to the tracheæ, while the irregular nuclei of the cylinder cells lie at a little distance away.

In his study of *Luciola* in 1886, Wielowiejski did not find the

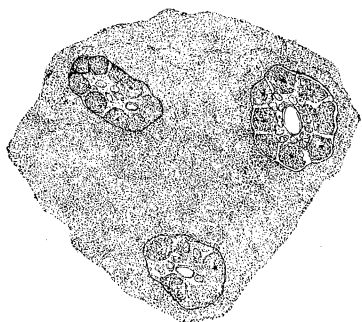


FIG. 10.—Section transverse to cylinder. Her-
man's fluid. $\times 235$.

cylinder a syncytium, as stated by Emery, but believed it to be composed of two elements, a nucleated epithelium immediately surrounding the trachea, and an outer layer belonging to the parenchyma. The latter he found generally separated from the trachea by the action of reagents, but still connected with the paren-

chyma cells. These observations would seem to be in agreement with the conditions found in *Photinus*.

The bulk of the cylinder is thus shown not to be of tracheal origin. The tracheæ pass into the cylinder cells, there dividing to form the tracheoles, so that the name "transition cells" is equally befitting here, although the structures to which it is applied cannot be considered homologous with those of the "capillary end-network" of the caterpillar. If the cylinders belong to the parenchyma, they are at least clearly distinguished from it by definite boundary lines. In some cases there might seem to be a transition between the cells of the dorsal layer and those of the cylinder, but the cylinder cells are much smaller than those of either the dorsal layer or the parenchyma. They retain their spherical shape much more than either of the others mentioned. It would seem probable that all

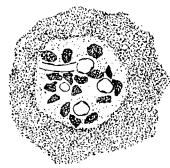


FIG. 11.—Section
through cylinder
showing epithel-
ium of trachea. \times
235.

three forms of cells are of the same origin and that their structural differences are due to difference in function.

Wielowiejski, from his work on *Lampyrus splendidula* believed in the possible transformation of the parenchyma cells of the ventral layer into the cells of the dorsal layer through the physiological effects of photogeny. Emery did not accept this theory, and after his study of *Luciola* and two American species of Lampyridæ, Wielowiejski himself ('89) stated that, for those forms at least, it was untenable.

The conditions in *Photinus* are such as to apparently preclude such an hypothesis. The two layers are distinctly separate in all preparations and the relative thickness of the layers is fairly constant. There is no indication of a transition between the two layers, nor is there any apparent difference in the thickness of the layers in material put up in early summer and in that taken at the close of the flying season. Still more important are the inherent differences in the two layers. In the dorsal layer there is a solid mass of polygonal cells, similar in form and size, and irregularly penetrated by tracheæ. In the ventral layer there is a distinct division into two elements, the cylinders enclosing the tracheal trees, and the parenchyma cells. The arrangement and distribution of the tracheæ of the ventral layer is strikingly regular. The parenchyma cells are extremely irregular, both in form and size. It would, therefore, seem difficult to suppose that the dorsal layer could grow at the expense of the ventral layer.

Several theories have been offered as to the origin of the photogenic tissue. Kölliker ('57) regarded the light organs as "nervous apparatus." Owsjannikow ('68) thought them of epithelial origin. Wielowiejski ('86) suggests their derivation from the "kleine Ænocyten" which he finds absent in the photogenic species of Coleoptera. The most general view, however, is that the photogenic tissue is differentiated fat body. This is upheld by a general similarity in structure, position and cell content. It has been already noted that granules exactly similar in appearance to the urate crystals of the dorsal layer have been found in the fat body near the light organs.

A question of this character could be settled only by onto-

genetic study of the photogenic tissue. This has not been attempted, so far as I can learn, by anyone besides Dubois ('98). He has studied the development of both *Lampyris noctiluca* and *Pyrophorus noctilucus* from the earliest stages. The eggs were found to be luminous even before they were laid, so that the light was transmitted in unbroken continuity from one generation to the next. Dubois followed the development of the light organs through all the different changes occurring from the beginning of segmentation to the emerging of the adult insect, and his observations led him to state definitely that the photogenic tissue is derived directly from the underlying hypodermis, by a proliferation of these cells. He also states that in the development of the organs a transformation takes place in the protoplasm of the cells, the older cells toward the upper surface of the light organs becoming filled with opaque, chalky granules. The younger cells, in which this transformation has not taken place, constitute the parenchyma.

While these results are not in line with previously accepted ideas in regard to the derivation of the light organs, they are based upon the only kind of study which can determine the problem. Apparently Dubois's work has not been generally accepted, and it needs verification by other workers. Nothing could be more profitable in our present state of knowledge than extensive and thorough study of the photogenic tissue throughout all its transformations.

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